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Chromatin immunoprecipitation (ChIP)

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An abbreviated version of this protocol was published in eLIFE in Nov 2016

POWERDRESS interacts with HISTONE DEACETYLASE 9 to promote aging in *Arabidopsis*

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Detailed protocol

1. Grind 2 g tissues into fine powder in liquid N2.
2. Transfer powder into 50 ml tube containing 25 ml Nuclear Isolation Buffer. Mix well.
3. Shack or rotate for 15 min at 4 degree (Try to avoid bubble).
4. Add 0.68 ml 37% formaldehyde (final concentration 1%). Incubate 15 min at RT with shacking or rotation (crosslink time may need to be optimized).
5. Stop crosslink by addition of 3.4 ml 1M Glycine (~125mM final).
6. Filter through 2 layers of Miracloth. Centrifuge at 3000 g for 20 min at 4 degree.
7. Discard supernatant and resuspend pellet with 1ml ChIP Buffer 2. Transfer to 1.5 ml tube. Centrifuge at 12,000 g for 10 min at 4 degree.
8. Discard supernatant. Resuspend pellet with 300 ul Nuclear Lysis Buffer. Keep on ice for 10 min. Add ChIP Dilution Buffer to 1 ml, and transfer to sonication tube. Sonication for 10 min with Plant Tissue Program (Covaris S220: Peak power:170; duty cycle:5.0; cycle burst:200). (Take 20-30 ul before and after sonication for DNA test)

(SDS is included in the Nuclear Lysis Buffer. If SDS is not compatible in the following steps, for example IP with FLAG M2 beads,we use MNase buffer to resuspend the pellet, DOUNCE with TIGHT for 20 times, then sonication for 20 min and followed by 1ul MNase digestion for 10 min at 37 degree. Then add EDTA and EGTA to 5mM to stop MNase digestion.)

9. Centrifuge at 12,000 rpm for 10 min. Transfer supernatant to new tube.
10. Add dilution buffer to 3ml (Dilute SDS to final 0.1%).Take 20 ul sample as “Input”. Keep in -20 degree.
11. Add 5 ug antibody (the amount depends on the quality of the antibody), incubate with 4 hr to O.N.
12. During incubation of the last step, prepare the Protein A/G beads. Transfer 40 ul beads to 1.5 ml tube, wash with ChIP Dilution Buffer or MNase Buffer.
13. Add the washed beads to the nuclear extract. Incubate for 2 hours.
14. Wash the beads-antibody-protein complex with 1 ml of the following buffer: Low Salt Buffer, High Salt Buffer, LiCl Buffer, TE buffer (If using the MNase Buffer, replace Low Salt Buffer with MNase Buffer, replace High Salt Buffer with MNase Buffer containing 500 mM NaCl). Each wash is performed twice: rinse the beads first, then wash for 5 min with rotation.
15. Discard wash buffer. Elute beads with 250 ul Elution buffer, shack at 65 degree with 1000 rpm shacking for 10 min.
16. Repeat elution once.
17. Combine elution. Add 20 ul 5M NaCl, shack at 65 degree with 800 rpm shacking for at least 6 hr. Remember to do the same for “Input” sample.
18. Add 10 ul 0.5 M EDTA, 20 ul 1 M Tris-HCl 7.0, 1 ul Protease K (20 ug/ul), 1 ul RNase (especially when use column to purify DNA) and incubate at 45 degree for 1 hr with 800 rpm shacking.
19. Add 500 ul Phenol/Chloroform/Isoamyl Alcohol (25:24:1), mix well. Centrifuge at 12,000 rpm for 10 min. Transfer ~400 ul upper layer to new tube, add 40 ul 3M NaAc, 2 ul glycogen (20 mg/ml), 1 ml EtOH. Keep at -80 O.N.
20. Spin at 13,000 rpm for 20 min. Wash with 70% EtOH. Dry, add 50 ul H_2O.

Nuclear Isolation Buffer
10 mM HEPES 8.0
1 M Sucrose
5 mM KCl
5 mM MgCl2
0.6% Triton X-100
0.4 mM PMSF
1X Mini-Complete Cocktail

ChIP Buffer 2
10 mM Tris-HCl 8.0
0.25 M Sucrose
10 mM MgCl2

1% Triton X-100
1 Mm EDTA
5 mM BME
1X Mini-Complete Cocktail

Nuclear Lysis Buffer
50 mM Tris-HCl 8.0
10 mM EDTA
1% SDS
0.1 mM PMSF
1X Mini-Complete Cocktail

ChIP Dilution Buffer
1.1% Triton X-100
1.2 mM EDTA
16.7 mM Tris-HCl 8.0
167 mM NaCl
0.1 mM PMSF
1X Mini-Complete Cocktail

MNase Buffer
50 mM Tris-HCl pH7.5
50 mM NaCl
5 mM MgCl2
5 mM CaCl2
10% Glycerol
0.1% NP-40
0.1 mM PMSF
Cocktail complete

Low Salt Buffer
20 mM Tris-HCl 8.0
150 mM NaCl
0.1% SDS
1% Triton X-100
2mM EDTA

High Salt Buffer
20 mM Tris-HCl 8.0
500 mM NaCl
0.1% SDS
1% Triton X-100
2mM EDTA

LiCl Buffer
10 mM Tris-HCl 8.0
250 mM LiCl
1% NP-40
1% Sodium deoxycholate
1 mM EDTA

TE Buffer
10 mM Tris-HCl 8.0
1 mM EDTA

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Zhong, X. and Chen, X. (2020). Chromatin immunoprecipitation (ChIP). Bio-protocol Preprint. bio-protocol.org/prep220.
2. Chen, X., Lu, L., Mayer, K. S., Scalf, M., Qian, S., Lomax, A., Smith, L. M. and Zhong, X.(2016). POWERDRESS interacts with HISTONE DEACETYLASE 9 to promote aging in *Arabidopsis*. eLIFE. DOI: 10.7554/eLife.17214